J. Glob. Innov. Agric. Sci.,2021, 9(2):85-88
ISSN (Online):2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/9.9465
http://www.jgiass.com

EFFECT OF DIFFERENT PLANT GROWTH REGULATORS ON *IN VITRO* REGENERATION OF MANGO: A REPORT

Muhammad Ahsan Qureshi[1,*], Waqar Shafqat[2], Safeer ud Din[1], Muhammad Kashif Raza[3], Naseem Sharif[4], Sufian Ikram[1] and Hafiz Amjad Ali Rana[5]

[1]Institute of Horticultural Sciences, University of Agriculture, Faisalabad-Pakistan; [2]Indian River Research and Education, Centre, University of Florida, Fort Pierce-34945, Florida, USA; [3]Date Palm Research Station, Jhang, Pakistan; [4]Horticultural Research Station, Sahiwal, Pakistan; [5]Institute of Agri. Extension, Education and Rural Development, University of Agriculture, Faisalabad-Pakistan.
[*]Corresponding author's e-mail: ahsanuaf3@gmail.com

Shoot tips of newly emerging mango shoots were used as an explant and cultured on MS media for direct shooting. Different plant growth regulators i.e. BAP, NAA and IAA in varying concentrations were added to basal MS media. Experiment was laid out according to Completely Randomized Design (CRD) with four treatments and each treatment was replicated thrice. Maximum shoot induction (55.56%) was observed with 1 mg/L of BAP. Maximum mortality (83.30%) was observed when maximum concentration of BAP (3 mg/L) was used. Minimum days (12.633) to induce shoots were observed on MS media supplemented with 1 mg/L of BAP. Maximum number of shoots (2.50) were recorded in MS media supplemented with 1 mg/L of BAP. Low concentration of NAA initiated roots earlier in regenerated shoots as compare to high concentrations of NAA and IAA. MS media supplemented with NAA (1 mg/L) took less days (21 days) to induce roots. The combination of auxins (MS+ NAA 3 mg /L + IAA 1 mg /L) proved the best for root induction (38.87%).
Keywords: *Mangifera indica*, tropical fruits, asexual propagation, plant tissue culture, micropropagation, phenolic exudation, acclimatization.

INTRODUCTION

Mango (*Mangifera indica* L.) is widely distributed in tropical and sub-tropical regions of the world. It is native to South East Asia and Indian subcontinent. The diversity, growth properties and nature of this fruit makes it unique and inimitable. Mango is popular for its flavor, color, juice, fragrance and nutritional properties. Pakistan is at fifth position in mango producing countries around the globe with annual production of 1.8 million tons and total area under mango cultivation is 171000 hectares. Pakistan has share of 3.9% in total mango production in the world (FAO, 2019).

Use of unhealthy seeds forming diseased seedlings and transmission of mango diseases from vegetative propagation material is among the major issues faced by mango industry (Memon, 2016). To maintain true to type nature in mono-embroynic mango cultivars, vegetative propagation is commonly employed which is time consuming and expensive method. In addition, there is great chance of transmission of plant borne diseases (Usman *et al.*, 2005). Unsuitability of current commercial cultivars to modern agricultural practices and stand still nature of conventional breeding approaches emphasize the importance in advancement of biotechnological approach in mongo cultivation and crop improvement (Krishna and Singh, 2007). *In vitro* propagation has become mandatory for commercial production of mango nursery as the most cultivars are highly susceptible to various plant borne diseases, and mass clonal propagation of desirable polyembronic rootstocks is solution for high rootstock demand (Tharanathan *et al.*, 2006). In contrast to other horticultural crops, *in vitro* regeneration in mango crop is most challenging. There are several factors such as phenolic exaudation, explant browning and media contamination which can ruin the whole culturing process (Pateña *et al.*, 2002; Al-Busaidi *et al.*, 2016). Raghuvanshi and Srivastava (1995) employed various blends of plant growth regulators such as cytokinin and auxin. Usually kinetin, BA, IAA and NAA are used in growth medium in order to attain numerous shoots of mango employing mature leaves as explants. For

Qureshi, M. A., W. Shafqat, S. ud Din, M. K. Raza, N. Sharif, S. Ikram and H. A. A. Rana. 2021. Effect of different plant growth regulators on in vitro regeneration of mango: a report. J. Glob. Innov. Agric. Sci. 9:85-88.

[Received 10 May 2021; Accepted 9 June 2021; Published 30 June 2021]

successful micropropagation, the *in vitro* regeneration protocol should be optimized for mango. Previous studies report that major shortcomings associated with regeneration of mango are death of tissues, media browning, phenolic release, contamination problems etc. These factors have the potential to reduce the success of *in vitro* regeneration practices (Karishna and Singh, 2007). The main objective of this study was to check the effect of growth regulators *in vitro* on shoot tip explants of mango.

MATERIALS AND METHODS

***Explant source and sterilization*:** Shoot tips were collected from soft and newly growing shoots of the Anwar Rataul plants from Fruit Plant Nursery. First, the shoot tips were washed with tap water to remove all the dirt and impurities and then the explants were immersed in 70% ethanol having 1-2 drops of Tween-20 detergent for 10 minutes. Further, sterilization of shoot tips was made using 1% mercuric chloride solution followed by 10% sodium hypochlorite. Explants were then rinsed with double distilled water and dipped in 1% PVP (Polyvinylpyrrolidone) and 200 mg/L ascorbic acid for 30 minutes for controlling the phenolic exaudation. This was followed by 3-4 times thorough rinsing with double distilled water. For controlling media browning 300 mg/L activated charcoal was also added in the growth medium.

***Media preparation and modification*:** Standard Murashige and Skoog (MS) media was used as basal medium with different combinations of growth regulators. Growth regulator stock solutions were prepared as per standard methods. Agar (0.8%) and 30 g sucrose were also incorporated to media. pH of the MS media was adjusted and maintained at 5.7 to 5.8. Media was autoclaved at 121°C for 15 minutes. Different concentrations of Benzylaminopurine (BAP) (1, 2 and 3 mg/L) and Indole Acetic Acid (IAA) (1, 2 and 3 mg/L) were added in media for shoot induction. For rooting, different concentrations of Naphthalene Acetic Acid (1, 2 and 3 mg/L) and Indole Acetic Acid (1, 2 and 3 mg/L) were added to standard MS media.

***Culture conditions*:** After surface sterilization, the explants were cultured in test tubes under laminar air flow hood cabinet. After inoculation, the cultures were transferred to the growth room with temperature 25±2°C and light with 16-hour day and 8-hour dark period.

Table 1. Media composition for *in vitro* propagation of mango.

S.No.	Shooting media	Rooting media
1	MS + BAP (1 mg/L)	MS + NAA (1 mg/L)
2	MS + BAP (1 mg/L) + IAA (3 mg/L)	MS + NAA (1 mg/L) + IAA (3 mg/L)
3	MS + BAP (2 mg/L) + IAA (2 mg/L)	MS + NAA (2 mg/L) + IAA (2 mg/L)
4	MS + BAP (3 mg/L) + IAA (1 mg/L)	MS+ NAA (3 mg/L) + IAA (1 mg/L)

***Statistical analysis*:** The experiment was laid according to Completely Randomized Design (CRD). Four treatments with three replications were used. Each replicate contained five cultures. Data was analyzed using Statistics 8.1 software

RESULTS AND DISCUSSION

***Shooting of in vitro grown explants*:** BAP showed better results for shoot induction as compared to IAA. Low concentration of BAP gave higher shoot induction percentage (Table 2). BAP 1 mg/L showed maximum shoot induction percentage (55.56%) while minimum shoot induction percentage (16.67%) was recorded on MS media supplemented with BAP 3 mg/L + IAA 1 mg/L. Similar results were also reported by Lad *et al*. (1997) who stated that BAP in low concentration is the best growth regulator for direct shoot induction of mango cultivars under *in vitro* conditions. BAP in combination with low doses of IAA can also induce direct shooing considerably. BAP 1 mg/L showed minimum mortality rate (44.31%) while MS media supplemented with maximum dose of BAP (3 mg/L) and IAA (1 mg/L) showed the highest mortality rate (83.33%). Our results are justified by the findings of Dewald *et al*. (1989a) who concluded that BAP can induce shooting in low concentrations. High concentrations of BAP had an antagonistic effect and cause mortality of explant. Low concentration of BAP gave the best results by inducting shoots in minimum days. Mango explants which were cultured on MS media supplemented with BAP 1 mg/L took 12.63 days for direct shoot induction while maximum days (15.40 days) for direct shoot regeneration were recorded on

Table 2. Effect of different PGR's on shooting of mango *in vitro*.

Treatments	Shooting (%)	Mortality (%)	Days to regenerate	Number of leaves	Shoot length (mm)
MS + BAP 1 mg/L	55.56±2.78^{a}	44.31±2.78^{d}	12.63±0.32^{c}	2.93±0.96^{ab}	1.37±0.19^{b}
MS + BAP 1 mg/L + IAA 3 mg/L	22.22±1.78^{c}	77.77±2.78^{b}	17.93±0.32^{a}	1.26±0.32^{b}	2.69±0.19^{ab}
MS + BAP 2 mg/L + IAA 2 mg/L	33.33±2.78^{b}	66.67±2.78^{c}	13.50±0.32^{b}	2.40±0.41^{ab}	2.58±0.19^{ab}
MS + BAP 3 mg/L + IAA 1 mg/L	16.67±1.33^{d}	83.33±2.78^{a}	15.40±0.32^{b}	3.53±0.41^{a}	3.51±0.19^{a}

The values are means ± Standard error; Means which are having different alphabet in a row or in a column are significant (P<0.05)

MS media supplemented with low concentration of BAP (1 mg/L) and high concentration of IAA (3 mg/L). Overall, MS + BAP 1 mg/L was the best treatment for shoot induction (Table 2). These results for days to induce shooting were justified by the results of Zimmerman (1993) that the explants respond best to MS medium supplemented with low concentrations of growth hormones. Low concentration of BAP enhances and fastens the regeneration capacity of explants on MS media. These results are further supported by the work of Misra and Datta (2011). Maximum number of leaves were sprouted at high concentration of BAP and less laves were sprouted at low concentration of BAP. Maximum (3.53) leaves were recorded When MS media was supplemented with maximum concentration of BAP (3 mg/L) and lower concentration of IAA (1 mg/L). Minimum (1.26) leaves were sprouted on MS + BAP 1 mg/L + IAA 3 mg/L. The illustrated results are against the finding of Misra and Datta (2011), who observed maximum expanded leaves at 0.5 µM BAP with combination of GA_3. One month after culturing to the shooting media, shoot length was counted for all four treatments (Table 2). Maximum shoot length was obtained at higher concentration of BAP. The treatment MS + BAP 3 mg/L + IAA 1 mg/L was the best treatment for enhancing the shoot length. Maximum shoot length (3.51 mm) was recorded when MS media was supplemented with high concentration of BAP (3 mg /L) and low concentration of IAA (1 mg/L). Minimum shoot length (1.37 mm) was recorded when BAP 1 mg/L was used. Our results are satisfactory in comparison to the work of Ara *et al*. (1999). They investigated that BAP is effective for shoot regeneration of mango in low concentration. Maximum shoot length was obtained by adding 1mg/L BAP to the MS media.

Figure 1. *In vitro* shoot induction and elongation in mango.

***Rooting of in vitro regenerated shoots*:** *In vitro* regenerated shoot cultures were then transferred to rooting media and data was recorded for rooting parameters. MS media supplemented with different concentrations of Naphthalene acetic acid and Indole acetic acid was used. Low concentration of NAA initiated roots earlier as compare to high concentration of NAA and IAA. Root induction in established shoot cultures took place within the time duration of 21 to 39 days (Fig. 2a). MS media supplemented with NAA 1 mg/L took less days (21 days) to induce roots. The combination of auxins having high concentration of NAA and low concentration of IAA (MS+ NAA 3 mg/L + IAA 1 mg/L) was the best for root induction (38.87%) (Fig. 2b). These results were in line with the work of Mathews *et al*. (1992) who worked on root induction of mango and concluded that high concentrations of NAA can be useful for root induction. They obtained very low survival percentage as in our results.

Maximum number of roots (3.0) were recorded on MS media supplemented with NAA 1 mg/L (Fig. 2c). These results were in resemblance with the work of Monsalud *et al*. (1995) who obtained maximum number of roots by applying 1 mg/L of NAA. Root length was highest on MS media supplemented with NAA 3 mg/L + IAA 1 mg/L (Fig. 2d). Concentration of different types of auxins in MS rooting helped in rooting of *in vitro* grown shoot cultures. Auxins are the primarily responsible growth hormones for rooting process. Monsalud *et al*. (1995) also conducted experiments on *in vitro* regeneration of mango. They applied varied concentrations of NAA and IAA in simple MS media. The best results were obtained by using 1 mg/L of NAA on simple MS media. The combinations of auxins also gave better results when combination of NAA and IAA were used.

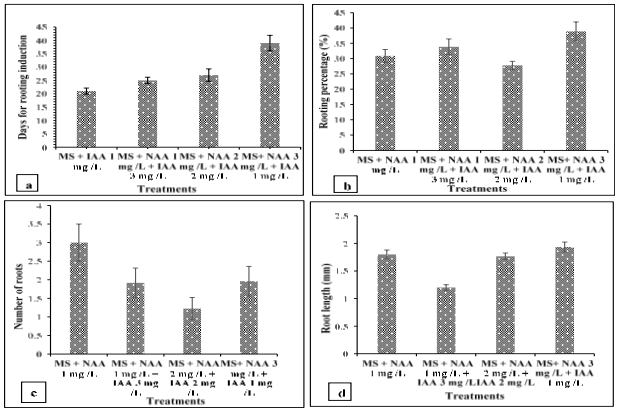

Figure 2. (a) Effect of growth regulators on days for root induction in mango (b) Effect of growth regulators on rooting percentage in mango (c) Effect of growth regulators on number of roots in mango (d) Effect of growth regulators on root length in mango.

***Conclusion*:** *In vitro* regeneration of mango has significance in mango breeding and improvement programs. Shoot induction from shoot tips was the highest on MS + BAP (1 mg /L). Low concentration of BAP had been more effective for shoot regeneration and other related parameters. For root initiation, the media with NAA 3 mg/L + IAA 1 mg/L provided the best results with the shortest days taken for rooting in addition to the highest rooting percentage and root length. The developed protocol further needs optimization

with special emphasis on acclimatization of regenerated plantlets.

***Author contribution statement*:** Qureshi MA: Conceived the idea, designed the study, and wrote the article; Shafqat W: Reviewing and editing; Din S: Helped in performing the experiment; Raza MK: Contributed in the framing and executing the research idea; Shairf N: Assisted in design layout and proofreading; Ikram S: Assisted in data collection and laboratory work; Rana HAA: Assisted in data collection and write up.

Funding: No funding was received for this study.

Acknowledgement: This study is the part of Master's Thesis. I acknowledged mu supervisor Dr. M.J. Jaskni for conducting me this study.

Conflicts of interest: The authors declare no conflict of interest.

REFERENCES

Al-Busaidi, K.T., M. Shukla, A.H. Al-Burashdi, G.S. Al-Blushi, M.H. Al-Jabri, B.S. Al-Kalbani and H.D. Al-Hasani. 2016. *In vitro* regeneration of mango (*Mangifera indica* L.) cv. Baramasi through nucellar embryogenesis. J. Hortic. For. 8:37-43.

Ara, H., U. Jaiswal and V.S. Jaiswal. 1999. Germination and plantlet regeneration from encapsulated somatic embryos of mango (*Mangifera indica* L.). Plant Cell Rep. 19:166-170.

Dewald, S.G., R.E. Litz and G.A. Moore. 1989a. Optimizing somatic embryo production in mango. J. Am. Soc. Hort. Sci. 114:712-716.

Dewald, S.G., R.E. Litz and G.A. Moore. 1989b. Maturation and germination of mango somatic embryos. J. Amer. Soc. Hort. Sci. 114:837-841.

FAO. 2014. FAOSTATdatabase/Production.Crops. Available online at http://apps.foa.org./lim500/nphwrap.pl

Gill, R., T. Senaratna and P.K. Saxena. 1994. Thidiazuron-induced somatic embryogenesis enhances viability of hydrogel-encapsulated somatic embryos of geranium. J. Plant Physiol. 143:726-732.

Jana, M.M., R.S. Nadgauda, K. Rajmohan and A.F. Mascarennhas. 1994. Rapid somatic embryogenesis from the nucelli of monoembryonic mango varieties. In Vitro Cell. Dev. Biol.- Plant 30:55-57.

Johnson, G.I., A.J. Mead, A.W. Cooke and J.R. Dean. 1991. Mango stem end rot pathogens-infection levels between flowering and harvest. Ann. Appl. Biol. 119:465-73.

Kabir, A., R. Islam, A. Haque, M. Khalekuzzaman and O.I. Joarder. 1994. Micropropagation of *Ziziphus mauritiana* from nodal segments of mature trees. Bull. Seric. Res. Bd. 5:5-8.

Khan, M.D. and A.H. Khan. 1960. Studies on malformation of mango inflorescence (West Pakistan). Punjab Fruit J. 23:247-258.

Kim, D.S. and S.P. Lee. 1988. Study on practical micropropagation of jujube cultivars through axillary bud culture. J. Kor. For. Soc. 77:445-452.

Krishna, H. and S.K. Singh. 2007. Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement—a review. Biotechnol. Adv. 25:223-243.

Krishna, H. and S.K. Singh. 2007. Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement—a review. Biotechnol. Adv. 25:223-243.

Lad, B.L., S. Jayasankar, F.A. Pliego-Alfaro, P.A. Moon and R.E. Litz. 1997. Temporal effect of 2,4-D on induction of embryogenic nucellar cultures, culture maintenance and maturation of 'Carabao' mango somatic embryos. In Vitro Cell. Dev. Biol. Plant. 33:253–257.

Memon, N.A. 2016. Mango: Pakistan 4th Largest Producer in the World. Available online at https://www.foodjournal.pk/2016/March-April-2016/PDF-March-April-2016/Exclusive-article-Mango.pdf

Misra, P. and S.K. Datta. 2001. Direct differentiation of shoot buds in leaf segments of white marigold (*Tagetes erecta* L.). In Vitro Cell. Dev. Biol.- Plant. 37:466-470.

Patena, L.F., L.R. Carlos-Refuerzo and R.C. Barba. 2002. Somatic embryogenesis and plantlet regeneration in mango (*Mangifera indica* L.). In Vitro Cell. Dev. Biol.- Plant 38:173-177.

Purseglove, J.W. 1972. Mangoes of India. Acta Hort. 24:107-174.

Raghuvanshi, S.S. and A. Srivastava. 1995. Plant regeneration of *Mangifera indica* using liquid shaker to reduce phenolic exudation. Plant Cell Tiss. Org. Cult. 41:83-85.

Sharma, R.R. and S.K. Singh. 2002. Etiolation reduces phenolic content and polyphenol oxidase activity at the pre-culture and in vitro exudation of phenols from mango explants. Trop. Agric. 79:94-98.

Singh, S.K., H.C. Sharma, S.P. Singh and P.B.K. Kishore. 1991. Callus induction and root initiation from explants of zygotic embryos in mango hybrids. Plant Tissue Culture and Biotechnology: emerging trends. Proc. of Sym. pp.129-33.

Tharanathan, R.N., H.M. Yashoda and T.N. Prabha. 2006. Mango (*Mangifera indica* L.), "The king of fruits"—An overview. Food Rev. Int. 22:95-123.

ud Din, S., W. Shafqat, M.A. Qureshi, A.R. Saleem, N. Sharif, M.K. Raza, S. Ikram and M. Iqbal. 2020. *In vitro* regeneration of litchi (*Litchi chinensis* Sonn.) through shoot bud culture. J. Glob. Innov. Agric. Soc. Sci. 8:141-146.

Usman, M., M.J. Jaskani, M.Y. Rehman, B. Fatima and I.A. Khan. 2005. *In vitro* regeneration of monoembryonic mango cultivars. Int. J. Biol. Biotechnol. 2:23-27